3131

ONCOSTREAMS: NOVEL DYNAMICS PATHOLOGICAL MULTICELLULAR STRUCTURES INVOLVED IN GLIOBLATOMA GROWTH AND INVASION

Andrea Comba[1], Patrick Dunn, Anna E Argento, Padma Kadiyala, Sebastien Motsch, Phillip Kish, Alon Kahana, Daniel Zamler, Karin Muraszko, Maria G Castro and Pedro R Lowenstein
[1]University of Michigan

OBJECTIVES/SPECIFIC AIMS: Oncostreams represent a novel growth pattern of GBM. In this study we uncovered the cellular and molecular mechanism that regulates the oncostreams function in GBM growth and invasion. METHODS/STUDY POPULATION: We studied oncostreams organization and function using genetically engineered mouse gliomas models (GEMM), mouse primary patient derived GBM model and human glioma biopsies. We evaluated the molecular landscape of oncostreams by laser capture microdissection (LCM) followed by RNA-Sequencing and bioinformatics analysis. RESULTS/ANTICIPATED RESULTS: Oncostreams are multicellular structures of 10-20 cells wide and 2-400 μm long. They are distributed throughout the tumors in mouse and human GBM. Oncostreams are heterogeneous structures positive for GFAP, Nestin, Olig2 and Iba1 cells and negative for Neurofilament. Using GEMM we found a negative correlation between oncostream density and animal survival. Moreover, examination of patient's glioma biopsies evidenced that oncostreams are present in high grade but no in low grade gliomas. This suggests that oncostreams may play a role in tumor malignancy. Our data also indicated that oncostreams aid local invasion of normal brain. Transcriptome analysis of oncostreams revealed 43 differentially expressed (DE) genes. Functional enrichment analysis of DE genes showed that "collagen catabolic processes", "positive regulation of cell migration", and "extracellular matrix organization" were the most over-represented GO biological process. Network analysis indicated that Col1a1, ACTA2, MMP9 and MMP10 are primary target genes. These genes were also overexpressed in more malignant tumors (WT-IDH) compared to the less malignant (IDH1- R132H) tumors. Confocal time lapse imagining of 3D tumor slices demonstrated that oncostreams display a collective motion pattern within gliomas that has not been seen before. DISCUSSION/SIGNIFICANCE OF IMPACT: In summary, oncostreams are anatomically and molecularly distinctive, regulate glioma growth and invasion, display collective motion and are regulated by the extracellular matrix. We propose oncostreams as novel pathological markers valuable for diagnosis, prognosis and designing therapeutics for GBM patients.

3024

Osteocyte-derived CXCL12 is Essential for Load-Induced Bone Formation in Adult Mice

Pamela Cabahug Zuckerman[1], Chao Liu, Emily Fang and Alesha B Castillo
[1]New York University - H+H Clinical and Translational Science Institute

OBJECTIVES/SPECIFIC AIMS: Our aim is to test whether osteocyte-specific CXCL12 expression is critical to exercise-driven bone formation. METHODS/STUDY POPULATION: All procedures were approved by the NEW YORK UNIVERSITY Institutional Animal Care and Use Committee. We generated male and female mice in which CXCL12 was deleted from OCYs (CXCL12ΔOCY) by crossing CXCL12 floxed mice and 10kb DMP1-Cre transgenic mice (gifts from Drs. Geoffrey Gurtner and Lynda Bonewald, respectively). The 10kb DMP1-Cre has been shown to be robustly expressed in odontoblasts and OCYs, with little to no activity in cells from non-mineralized tissues (Lu+ J Dent Res 2007). Growing male and female mice (n=3-8/group) were given fluorochrome labels every two weeks between 4-16 weeks of age, to monitor the role of CXCL12 during development. A second group, of adult 16-week-old mice (n=5/group), were subjected to tibial axial cyclic loading (1200με, 2Hz, 120cycles, 3days/wk for 2 wks) (Liu+ Bone 2018). Basal and load-induced periosteal (Ps) and endosteal (Es) mineralizing surface (MS/BS, %), mineral apposition (MAR, μm/day) and bone formation rates (BFR/BS, μm3/μm2/year) were calculated (Dempster+ JBMR.2013) at mid-length. RESULTS/ANTICIPATED RESULTS: No significant differences were detected in basal bone formation during development. However, relative load-induced Ps MAR (rMAR) was reduced by 50% in female (p=0.02) and 75% in male (p=0.002) CXCL12ΔOCY mice; and similarly, Ps rBFR/BS was reduced by 50% in female (p=0.01) and 70% in male (p=0.001) CXCL12ΔOCY mice (Figure 1). Es bone formation was not affected by CXCL12 deletion. DISCUSSION/SIGNIFICANCE OF IMPACT: In summary, osteocyte-specific CXCL12 expression plays a critical role in exercise-driven periosteal new bone formation, suggesting that CXCL12 signaling may positively regulate osteogenic differentiation and/or mature osteoblast function. Further underlying mechanisms are currently being explored. Thus, osteocyte-specific CXCL12 signaling may be a promising target to enhance load-induced bone formation in patients with compromised ability to form new bone.

3332

Overexpression of CD44 is involved in the development of the early endometriotic lesion in a xenograft model

Jennifer Knudtson[1], Jessica McLaughlin, Marlen Tellez Santos, Rajeshwar R Tekmal and Robert Schenken
[1]University of Texas Health Science Center San Antonio

OBJECTIVES/SPECIFIC AIMS: Previously, we showed decreased development of endometriotic lesions in CD44 knockout mice compared to control.(1) CD44 has 10 different variants and a standard form. Menstrual endometrial cells (MECs) from women with endometriosis have increased adhesion and also express higher levels of CD44 variant 6 (v6) than v3, compared to MECs from women without endometriosis. (2) Here, we assessed the effects of CD44 standard (CD44s), CD44v3 and CD44v6 overexpression (OE) on immortalized human endometrial epithelial (iEECs) and stroma cells (hESCs) in vivo attachment in a nude mouse xenograft model. 1. Knudtson JF, Tekmal RR, Santos MT, et al. Impaired Development of Early Endometriotic Lesions in CD44 Knockout Mice. Reproductive sciences (Thousand Oaks, Calif.). 2016;23(1):87-91. 2. Griffith JS, Liu YG, Tekmal RR, Binkley PA, Holden AE, Schenken RS. Menstrual endometrial cells from women with endometriosis demonstrate increased adherence to peritoneal cells and increased expression of CD44 splice variants. Fertility and sterility. 2010;93(6):1745-1749. METHODS/STUDY POPULATION: Overexpression of CD44s, CD44v3 and CD44v6 was carried out using lipofectamine and their expression verified with qRT-PCR in iEEC and hESCs. Nude mice, 8-10 week old, were injected with estrogen 1 week prior to injection of iEECs and hESCs (n=7 per group). The cells were counted after transfection and at least 300,000 iEECs and 300,000 hESCs were injected per mouse. The transfected cells were tagged with cell tracker red (iEECs) and green (hESCs). Forty-eight hours after injection into the

xenograft, the mice were sacrificed. The cells were counted using fluorescent stereo microscopy (FSM). Percent attachment was calculated based on the number of cells visualized by FSM divided by the number of transfected cells injected. Unpaired student t-test was performed to analyze differences in the percent attachment of the cells. RESULTS/ANTICIPATED RESULTS: The majority of cells were attached to the peritoneum. There was increased attachment of hESCs with OE of CD44v6 compared to control (p=0.03). CD44v6 OE did not change attachment of iEECs. There was no difference in attachment in iEECs or hESCs with OE of CD44s or CD44v3. DISCUSSION/SIGNIFICANCE OF IMPACT: Overexpression of CD44v6 increases attachment of ESCs to PMCs in an in vivo xenograft model. Menstrual endometrial cell type and CD44 variants play a complex role in the development of the early endometriotic lesion.

3468

Predictive biomarkers of platinum-based chemotherapy response in Puerto Rican Hispanics with high-grade serous ovarian cancer.

Jeyshka M. Reyes-Gonzalez[1], Sharee Umpierre and Pablo E. Vivas-Mejia
[1]University of Puerto Rico-Medical Sciences Campus

OBJECTIVES/SPECIFIC AIMS: High-grade serous ovarian carcinoma (HGSOC) is the most common and malignant histological subtype of epithelial ovarian cancer. While the majority of HGSOC patients initially respond to platinum-based chemotherapy, they often present with recurrent chemoresistant disease, which is extremely fatal. Therefore, there is an urgent need to identify predictive biomarkers of platinum response and to develop rational, targeted therapies to improve the outcome of patients with HGSOC. The objectives of the present study are to profile and assess the clinical significance of MYC network dysregulation in HGSOC. METHODS/STUDY POPULATION: We will conduct a retrospective cohort study of Puerto Rican Hispanics with HGSOC who underwent surgery followed by platinum-based chemotherapy at clinical institutions in Puerto Rico. Medical records, pathology reports, and cancer registries will be reviewed to extract data on clinicopathological features, disease recurrence, and death. For eligible patients, formalin-fixed, paraffin-embedded (FFPE) tissue samples will be processed and analyzed by quantitative Real Time PCR (qRT-PCR) and immunohistochemistry (IHC). RESULTS/ANTICIPATED RESULTS: Expression levels of MYC and MYC-related molecules are expected to correlate with clinicopathological features and prognosis of HGSOC. DISCUSSION/SIGNIFICANCE OF IMPACT: The identification and validation of clinically-relevant alterations in HGSOC, such as dysregulation of the MYC network, will be crucial to guide therapy regimen, maximize clinical benefit, and improve patient outcome.

3506

PRMT5 is a novel therapeutic target to enhance radiation therapy for cancer treatment

Jake L Owens[1], Elena Beketova, Samantha Tinsley, Andrew Asberry, Xuehong Deng and Chang-Deng Hu
[1]Indiana University School of Medicine

OBJECTIVES/SPECIFIC AIMS: Prostate cancer is the second leading cause of cancer-related death among men in the U.S. and over half of all prostate cancer patients receive radiation therapy (RT). RT induces double-strand breaks (DSBs) in DNA which are lethal to cells if not repaired. While potentially curative, 10% of low-risk patients and 50% of high-risk patients treated with RT still experience tumor recurrence. Thus, identification of novel therapeutic targets to enhance RT will likely reduce prostate cancer mortality. The only clinical approach to enhance RT is androgen deprivation therapy, which targets androgen receptor (AR) signaling; however, its use is limited due to systemic side effects. We recently reported that PRMT5 epigenetically activates AR which led us to investigate if targeting PRMT5 sensitizes prostate cancer to RT. The goal of this project is to determine if PRMT5 is a therapeutic target for prostate cancer radiosensitization and analyze its mechanistic role in response to radiation. METHODS/STUDY POPULATION: To evaluate if targeting PRMT5 may sensitize prostate cancer cells to radiation, we performed a clonogenic assay of irradiated cells. To determine if PRMT5 is required for repair of radiation-induced DSBs, we performed foci analysis via immunocytochemistry. We then used RNA-seq, qPCR, western blot, and ChIP to evaluate a potential epigenetic role of PRMT5 in activating the expression of genes critical to DSB repair. To extend our findings, we analyzed clinical data from around 18,000 of cancer patients encompassing 43 cancer types to assess if PRMT5 expression correlates with the expression of its putative target genes. RESULTS/ANTICIPATED RESULTS: Targeting PRMT5 sensitizes prostate cancer cells to radiation independently of AR status. RNA-seq analysis revealed putative PRMT5 target genes including several involved in DSB repair and G2 arrest. Mechanistically, PRMT5 functions as a master epigenetic activator of DNA damage response (DDR) genes: PRMT5 maintains the basal expression of several DDR genes including BRCA1, BRCA2, and RAD51 and is recruited upon radiation to DDR gene promoters to activate their expression via histone methylation. Targeting PRMT5 decreases expression of these genes at the protein level and hinders repair of radiation-induced DSBs in multiple cancer and non-cancer cell types. Clinically, PRMT5 expression positively correlates with the expression of these DDR genes across all 43 cancer types analyzed. DISCUSSION/SIGNIFICANCE OF IMPACT: PRMT5 acts as a master epigenetic activator of genes involved in DDR and is critical for cells to survive radiation treatment. Importantly, PRMT5 epigenetically activates multiple genes that encode for well-characterized core repair proteins involved in HR (RAD51, RAD51AP1, RAD51D, BRCA1 and BRCA2) and NHEJ (NHEJ1, Ku80, XRCC4, and DNAPKcs), which may explain why PRMT5 is essential to repair IR-induced DSBs in several cell lines. As PRMT5 is overexpressed in many human cancers and its overexpression correlates with poor prognosis, our findings suggest that more efficient DSB repair via PRMT5 overexpression in these cancers may confer survival advantages particularly following DNA damaging treatments. Lastly, because targeting DSB repair is a clinically validated therapeutic approach for cancer treatment, our findings also suggest that PRMT5 targeting may be explored as a monotherapy or in combination therapy with radiation therapy or chemotherapy for cancer treatment.

3204

Renin-Angiotensin System Inhibitors Do Not Improve Survival in Fibrillin-1 Hypomorphic Mice with Established Aortic Aneurysm

Mary Burchett Sheppard[1], Jeff Zheying Chen, Debra L. Rateri, Jessica J. Moorleghen, Mackenzie Weiland and Alan Daugherty
[1]University of Kentucky Center for Clinical and Translational Science

OBJECTIVES/SPECIFIC AIMS: Drugs to attenuate aortic growth are usually not initiated in patients with Marfan syndrome until